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Rush Quillwort (*Isoetes junciformis*, sp. nov.), a New Pteridophyte from Southern Georgia

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ABSTRACT.—A previously undescribed pteridophyte, *Isoetes junciformis*, is reported from Tift County, Georgia. This new tetraploid appears to be a rare endemic of the upper Coastal Plain region. It is suspected to be an allopolyploid, possibly arising from the hybrid between *I. flaccida* and *I. melanopoda*.

Quillworts (Isoetaceae) have traditionally been considered rare pteridophytes in the Coastal Plain region of southern Georgia. Snyder and Bruce (1986) reported only the diploids, *Isoetes engelmannii* A. Braun and *Isoetes flaccida* Shuttlew. ex A. Braun, from this area. They also noted a number of suspected sterile hybrids, viz., those from southern Georgia listed in Boom (1982). All of those specimens of suspected hybrids, however, have been revised to one or another species, as have the southern Georgia specimens of *I. engelmannii* listed in Snyder and Bruce (1986). Despite these reductions, the total number of quillwort taxa in southern Georgia has increased dramatically in recent years.

Luebke (1992) described two new hexaploid species, *Isoetes georgiana* Luebke and *I. boomii* Luebke, as endemics of the upper coastal plain. The former subsequently has been found to be locally common in several southern Georgia watersheds (Brunton and Britton, 1996b). The recently described tetraploid, *I. hyemalis* D.F. Brunt. (Brunton et al., 1994), has now been recorded from three counties in southwestern Georgia, from a turn-of-the-century collection (Brunton and Britton, 1996a) and through contemporary field work (J. R. Allison, pers. comm.). Another newly described tetraploid, *Isoetes appalachiana* D.F. Brunt. & D.M. Britton (Brunton and Britton, 1997), was also found in two counties in southern Georgia during field investigations by R. Carter and J. R. Allison (pers. comm.). Finally, collections from 1949 have been seen recently that confirm the occurrence of *Isoetes melanopoda* Gay & Dur. from Miller County in southwestern Georgia (Big Drain below Babcock Pond, *Thorne & Muenscher 9114* [GA, PH]). The identities of other *Isoetes* populations in southern Georgia have yet to be settled, indicating that the discovery of other taxa, including previously undescribed species, is possible.

In this paper we report an addition to the list of *Isoetes* in southern Georgia. The taxon described below is from a population first discovered in 1970 by

W. R. Faircloth and apparently represents a rare, previously unknown coastal plain endemic.

MATERIALS AND METHODS

As part of ongoing systematic studies of *Isoetes* in North America, approximately 1,500 herbarium specimens of *Isoetes* have been studied from the southeastern United States deposited at CAN, DFB (D. F. Brunton personal herbarium), DUKE, FLAS, FSU, GA, MICH, NCSC, NCU, NYS, OAC, PH, PSU, UNA, UNCC, USCH, USF, VDB, VPI, and VSC, as well as selected specimens from GH, MO, NY, and US. Specimens that could not be attributed to established taxa were detected during these herbarium studies and, where possible, site investigations of the populations of origin were undertaken. Scanning electron microscope (SEM) photographs of selected megaspore and microspore samples were taken using the standard methods of Britton and Brunton, (1989, 1992).

Microspores were measured in Euparol, as described by Britton (1991). Megaspore widths (to the outer edges of spore ornamentation) were measured at a magnification of 40× or 50× on SEM stubs or in sporewells (Brunton, 1990) using a binocular stereo microscope equipped with an ocular micrometer.

Chromosome counts were obtained from living material of *Brunton & McIntosh 11,818* and *Brunton & McIntosh 13,525* from Chula, Tift Co., GA. Plants were grown in distilled water in a growth cabinet. The developing root tips were excised and pre-treated in aqueous paradichlorobenzene (PDB) at room temperature for four hours. Then, they were washed in distilled water, fixed in acetic alcohol (3:1 absolute ethyl alcohol to glacial acetic acid) for 30 minutes or more, hydrolysed in Warmke's solution (1:1 concentrated HCl to absolute ethyl alcohol) for 7–10 minutes at room temperature, and stained in leucobasic fuchsin (Feulgen) for two hours. The meristems were squashed under a cover glass in 45% acetocarmine stain and examined under a light microscope.

RESULTS

We could not attribute a collection identified as *Isoetes flaccida* from Chula, Tift County, Georgia (*W.R. Faircloth 6690*), to any known *Isoetes* taxa. Cytological investigation of living plants obtained from the site indicated that these plants are tetraploid ($2n = 44$), a chromosome number previously undetected in *Isoetes* in Georgia. The population also proved to be morphologically distinct from the two newly described tetraploids, *I. appalachiana* and *I. hyemalis,* which have been discovered recently in this region. The following describes the morphological characteristics of the unnamed tetraploid from Chula, Georgia.

GROSS MORPHOLOGY.—The Chula tetraploid is a large quillwort, with ± erect,

FIG. 1. *Isoetes junciformis* plant (arrow) among graminoids (Tift County, GA, 4 May 1994)

dull, pale green to grayish green leaves reaching lengths of 35–40 cm (Fig. 1). Although many quillworts bear a superficial resemblance to newly developing graminoid plants, the Chula tetraploid appears remarkably like the young *Juncus* or sedges with which it associates (Fig. 2). Its pale-colored leaves are white to hyaline at the base. Many young plants have a distinctly pinkish purple wash through the pale basal 2–3 cm of their leaves.

The oval, heavily short-brown-streaked sporangia are each topped by a relatively large, narrowly-triangular, blunt-tipped and delicate ligule. Fresh, intact ligules (mostly on immature leaves) reach 35–40% the length of the sporangium. The velum extends from the base of the ligule across ca. 40% of the sporangium (Fig. 3).

The rounded or two-lobed corm supports a dense mass of round, hollow, relatively straight, grayish brown roots that branch dichotomously near the ends. The roots become flattened and a darker dusky brown color upon drying.

MEGASPORE SIZE AND MORPHOLOGY.—Well-formed, intact megaspores average ca. 460 μm in diameter. Megaspores have a glazed, porcelain-like surface. The proximal hemisphere is densely covered with low, irregular protuberances and short, broad mounds (Fig. 4a). In lateral view, a broad band of subdued, obscure mounds usually can been seen bordering the distal side of the equatorial ridge (Fig. 4b). The distal hemisphere of well-formed megaspores is prominently ornamented with a broken-reticulate pattern of low, broad, interconnecting ridges (Fig. 4c).

The megaspores of some plants of the Chula tetraploid are variable in size

FIG. 2. Young *Isoetes junciformis* (arrow) with an immature rush (*Juncus* sp.) (Tift County, GA, 19 Mar 1998).

and ornamentation. Some of the smaller spores (430–440 μm) are misshapened and with a dense, broken-reticulate ornamentation pattern. The variable, somewhat polymorphic megaspore ornamentation observed on these specimens resembles the condition observed in primary sterile hybrids. Despite careful searching of the site and its vicinity in 1994 and 1998, however, no other taxa have been discovered, although *Isoetes georgiana* was reported at "*many different areas of swamp forest*" in the adjacent floodplain of Whiddons Mill Creek (*Musselman & Allison 96–207* [ODU]). This is in contrast to finding at least one and usually both of the putative parents growing with *all* confirmed hybrid populations in North America (Britton 1991; Britton and Brunton, 1996; Musselman et al. 1997).

Stronger evidence yet against a hybrid origin of the Chula population is provided by the determination of tetraploid chromosome counts even from plants with some megaspores of variable size and polymorphic ornamentation (e.g., *Brunton & McIntosh 13,525*) (see also Origins, below). Accordingly, because all plants in the Chula population are tetraploids whether they produce either uniform or some polymorphic megaspores, we believe that megaspore variation in this case is most likely the result of developmental polymorphism. It is likely environmentally induced in the Chula tetraploid, as it appears to be in many populations of other southeastern quillworts that also develop in ecologically stressful situations. Examples of such taxa include the ephemeral

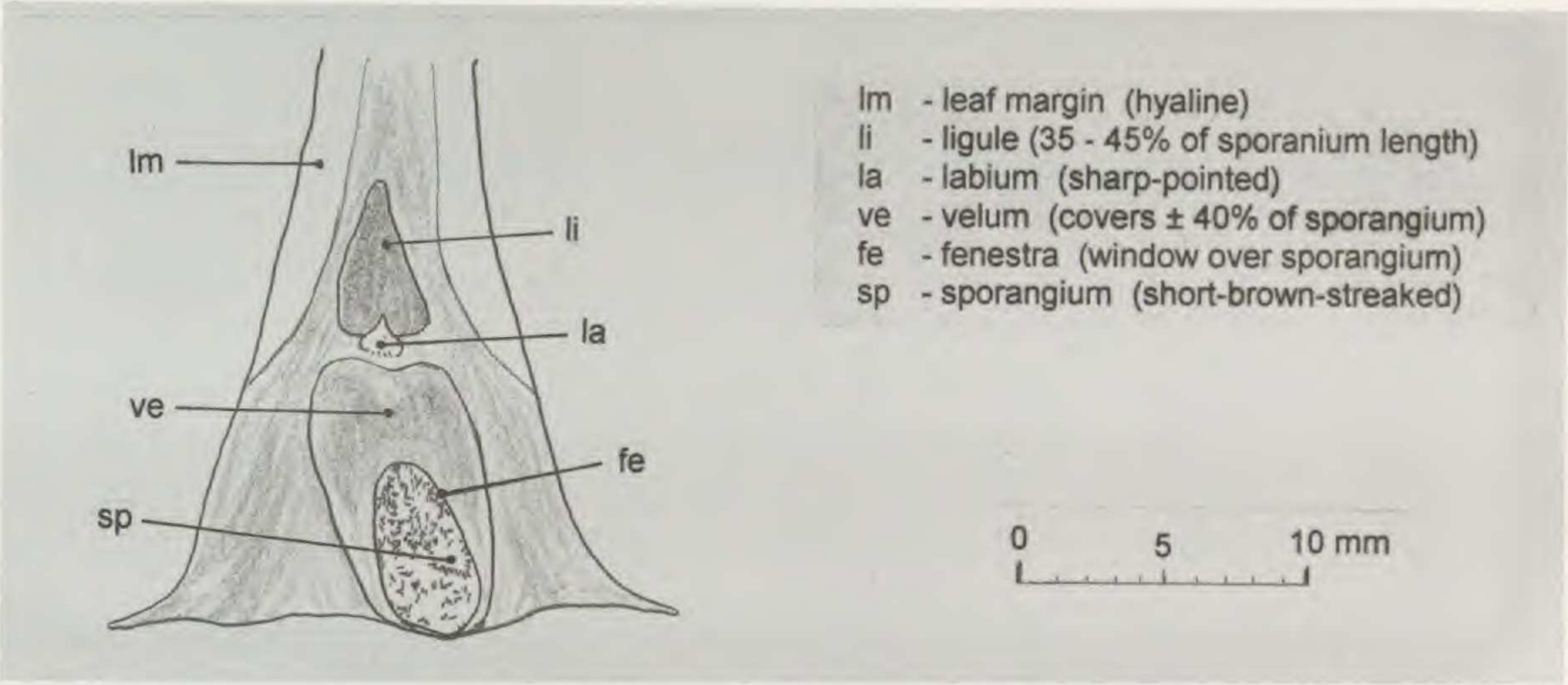

FIG. 3. Basal adaxial side of megasporophyll of *Isoetes junciformis* (tracing from *Brunton & Crins 11,848* [DFB]).

wetland species, *I. melanospora* Engelm., *I. piedmontana* (N. Pfeiff.) C.F. Reed, *I. virginica* N. Pfeiff., and *I. melanopoda*.

MICROSPORE SIZE AND MORPHOLOGY.—The oval microspores average approximately 30 μm in length (Fig. 4d). The microspores are strongly spinulose, being densely covered with fine-tipped but relatively broad-based spines on all surfaces.

SITE ECOLOGY.—The Chula tetraploid is found in a low, seasonally flooded swale at the base of a northwest-facing sandy slope (Fig. 5). The plants are found most commonly as scattered individuals in areas of the swale that remain most deeply flooded for the longest period. They are usually growing somewhat in isolation of associated graminoid vegetation. The quillworts grow with their corms at the bottom of a 3–5 cm deep layer of silty-clay (pH ±6.0) with their roots extending beneath that into coarse sand. No plants were found in those portions of the swale where the substrate lacks a substantial silt or clay component.

The site was submerged by 15–30 cm of quietly flowing water during the height of spring floods in March 1998 (pers. obs.). In recent years (1994–1997), the site was virtually dry by early May. It occupies a narrow intermediate zone between the adjacent upland area and the outer edge of a mature, deciduous floodplain swamp forest dominated (at its edge) by red maple (*Acer rubrum* L.) and sweet gum (*Liquidamber styraciflua* L.). The original character of the upland vegetation is unknown as the site was logged about 1990, then planted with loblolly pine (*Pinus taeda* L.) seedlings.

Plants become more difficult to distinguish from associated graminoid vegetation as the swale dries out; the site is typically dominated by graminoid vegetation by May or June (W. R. Faircloth, pers. comm.; pers. obs.). The few small plants detectable in August 1994 among the relatively dense graminoid vegetation possibly represent new growth responding to periodic mid-summer

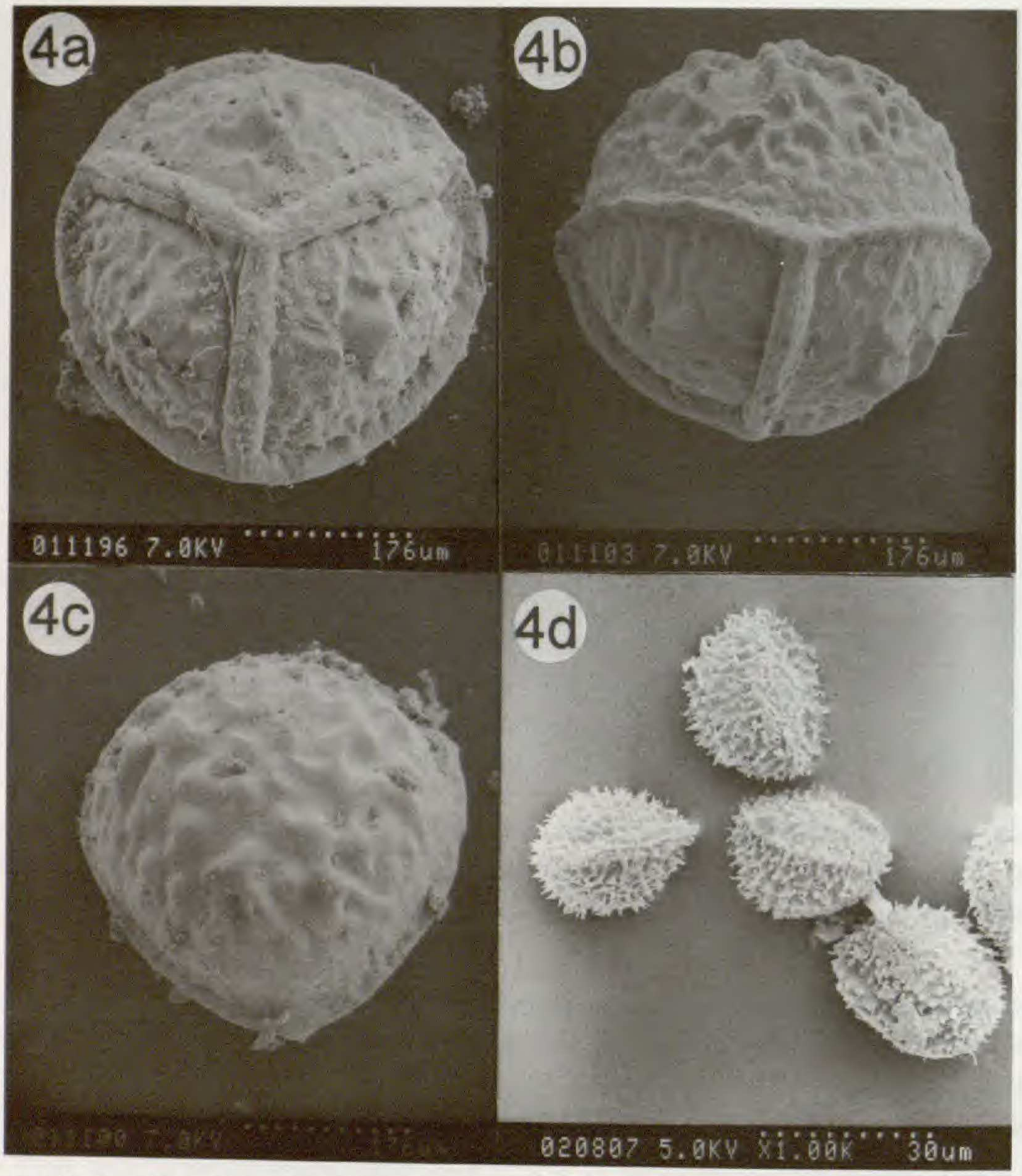

FIG. 4. *Isoetes junciformis* spores (*Brunton & Crins 11,848* [OAC]). a) proximal view of megaspore; b) lateral view of megaspore; c) distal view of megaspore; d) microspores.

flooding. Although pine saplings in the adjacent plantation have dramatically increased in height over this period, it is not apparent that any significant change in the amount of light reaching the quillwort site has occurred.

DISTRIBUTION AND STATUS.—More than fifty plants were observed along a 30–40 m length of the quillwort swale in March and May 1994. Approximately 75–100 plants were observed here during the height of the March 1998 flooding.

No other populations of the Chula tetraploid have been confirmed. Specimens from near Leary, Calhoun County, in southwestern Georgia (*Kral* s.n., 11 May 1977, VDB 158307, VDB 158308) appear to have comparable morphological characteristics to the Chula tetraploid, including megaspore and micro-

FIG. 5. *Isoetes junciformis* site in seasonally flooded swale between riparian swamp forest (right) and cleared (formerly forested) upland area (left) (Tift County, GA, 19 Mar 1994).

spore size and ornamentation, but chromosome counts from plants of the Calhoun County population have not been made.

DISCUSSION

Sufficient morphological and cytological evidence has been gathered from live and preserved material to indicate that the Chula tetraploid represents a previously undescribed species. Accordingly, the following binomial is proposed:

Isoetes junciformis D.F. Brunt. & D.M. Britton, sp. nov.—TYPE: U.S.A. Georgia, Tift County, 7 km WSW of Chula, Whiddons Creek near Little River, 3 May 1994 *D.F. Brunton & W.J. Crins 11,848* (OAC; isotypes MICH, MIL, DFB).

Herba erecta, inucea; folia glauca, velum tegens ±40% sporangii, maculis bruneis maculati; megasporae ±460 μm ornatae iugis humilibus atque latis, inter se concurrentibus; microsporae ovales, echinatae, ±30 μm. Chromosomatum numerus $2n = 44$.

In the following summary description of *Isoetes junciformis,* features particularly helpful for its identification are in boldface. Robust (25–40 cm tall), amphibious, perennial herbs from a 1.5–2.5 cm wide, rounded to two-lobed corm with numerous round, hollow, gray-brown, mostly unbranched roots;

leaves **stiffly erect to somewhat reflexed,** pale, **grayish lime-green,** white to hyaline, basal 2–3 cm **lightly washed with pinkish-purple** (at least when young); sporangia oval, ca. 7.5 × 4.0 mm, hyaline to white surface densely short-brown-streaked; velum covering **38.9%** (SD 6.62%, N = 23) of sporangium; ligule delicate, narrowly triangular, obtuse, 35–40% the length of the sporangium; megaspores **458.9 μm** (SD 39.64 μm, N = 50) in diameter when well-formed; some variable in size and apparently developmentally polymorphic, with ornamentation of **low, broadly rounded ridges in a ragged, broken-reticulate pattern** and conspicuous broad to narrow band of subdued, obscure ornamentation bordering the distal side of the equatorial ridge; microspores oval, **densely spinulose** with broad-based, **fine-tipped spines on all surfaces**; 29.9 μm (SD 1.26 μm, N = 20) long; Cytology: $2n = 44$.

The epithet reflects the rush-like appearance of well-developed plants.

PARATYPES.—U.S.A. **Georgia.** Tift County, WSW of Chula, Whiddon's Mill Creek near its junction with Little River, *W.R. Faircloth 6690* (GA, VSC); *D.F. Brunton & K.L. McIntosh 11,818* (DFB, OAC); *D.F. Brunton & K.L. McIntosh 12,051* (DFB); *D.F. Brunton & K.L. McIntosh 13,525* (DFB, OAC).

SIMILAR SPECIES.—The diploid *Isoetes melanopoda* appears to be morphologically the most similar species to tetraploid *I. junciformis,* particularly with regard to their grayish green, stiffly-erect to reflexed leaves, often with a pinkish purple wash in the pale basal section. Indeed, *I. junciformis* looks like a robust *I. melanopoda* with atypically bold megaspore ornamentation and an exceptionally large velum. *Isoetes melanopoda,* however, has smaller megaspores (404 μm, N = 60), with a substantially more obscure megaspore ornamenation (moderately to densely covered in small, low tubercles or short, vermiform crests and mounds) and a velum coverage rarely exceeding 15%. The other swampland diploid in south Georgia, *I. flaccida,* also has smaller (391 μm, N = 45), typically more obscurely ornamented megaspores (sparely to densely low tuberculate or with short, irregular, vermiform ridges), dark green leaves, and a sprawling, flaccid stature. It also is characterized by an extensive (80–100%) velum coverage of the sporangium.

Of the possible tetraploids, *Isoetes hyemalis* can be discriminated from *I. junciformis* by its more tuberculate megaspore ornamentation, shorter (15–20%) velum coverage, and dull olive-green to dark green, strongly reflexed leaves (Brunton et al., 1994). Tetraploid *I. appalachiana* also exhibits a shorter (20–25%) velum coverage and has dull olive-green to dark-green, strongly reflexed leaves, as well as a high-walled, strongly reticulate megaspore ornamentation pattern (Brunton and Britton, 1997). *Isoetes louisianensis* Thieret, the tetraploid endemic of coastal plain swamp forests in southern Louisiana and adjacent Mississippi (Lark, 1996), exhibits an even more congested, high-walled and reticulate megaspore ornamentation patttern with a distinctive equatorial band of short spines. It has a relatively large velum coverage (±30%) approaching that of *I. junciformis,* but also has substantionally larger megaspores (± 530 μm).

ORIGINS.—Most if not all North American sexual polyploid *Isoetes* species are believed to represent allopolyploids, as has been demonstrated for *I. riparia*

(Taylor et al., 1985; Taylor and Hoot, 1997) and *I. appalachiana* (W.C. Taylor, pers. comm). Polyploid sterile primary hybrids are also known, such as hexaploids *Isoetes ×fairbrothersii* J. Montgom. & W.C. Taylor (Montgomery and Taylor, 1994) and *I. ×hickeyi* W.C. Taylor & Luebke (Taylor and Luebke 1988).

An allopolyploid origin for *I. junciformis* would most likely result from the doubling of the hybrid between two diploids (2x × 2x = sterile 2x hybrid; doubled = fertile 4x species). Of the known southeastern diploids, the morphologically most similar and likely progenitors for *I. junciformis* would be *I. flaccida, I. melanopoda,* or *I. engelmannii.* None of these taxa is presently known to occur within ca. 80 km of the Tift County site.

A combination of the wide (80–100%) velum character of *I. flaccida* with the narrow (10–15%) velum of *I. melanopoda,* as well as their similarly low tuberculate to vermiform megaspore ornamentation patterns, would likely result in a plant demonstrating a similar morphological appearance to that of *I. junciformis.* An *I. engelmannii* × *I. melanopoda* combination is not a candidate as this hybrid represents the origin of *I. louisianensis* (Taylor and Hoot, 1998). A hybrid combination involving *Isoetes engelmannii,* in any event, would be expected to demonstrate a more evenly reticulate megaspore ornamentation (Brunton and Britton, 1996c). Development of *I. junciformis* from the doubling of the as-yet undiscovered primary diploid hybrid, *I. flaccida* × *I. melanopoda,* therefore seems plausible.

An alternative explanation for the development of *I. junciformis* could be the formation of a fertile population from the sterile hybrid between a hexaploid and a diploid species (6x × 2x = sterile 4x; selection for fertility over time = fertile 4x species). An *I. georgiana* × *I. melanopoda* hybrid, for instance, would presumably have many of the characteristics expected of the sterile progenitor of *I. junciformis.* Evidence for the utilization of this evolutionary pathway, however, has not been established for any North American quillwort (cf., Taylor et al., 1993).

Regardless of its origins, *I. junciformis* constitutes an addition to the growing number of endemic vascular plants known from this small area of the Georgia Coastal Plain. These include such wetland/riparian species as *Rhynchospora solitaria* Harper (Sorrie, 1998), and *I. georgiana* and *I. boomii* (Luebke, 1992; Brunton and Britton, 1996b).

As noted above under the Megaspore Size and Morphology section, available evidence indicates that *I. junciformis* is not a primary sterile hybrid. In addition to uniformly tetraploid chromosome counts being obtained from the Chula population, a wide range of plant sizes is evident within that population, indicating that on-going, *in situ* reproduction is taking place. With no other *Isoetes* taxa being found within the Chula population, this constitutes strong evidence that sexual reproduction is occurring there.

FURTHER RESEARCH.—The determination of the morphological characteristics and taxonomic significance of this population has been complicated by the rarity of living and preserved material. Some expressions of the variation observed in spore morphology, for example, are represented by only one or two known specimens. Morphological and cytological examination of living material of *I. flaccida* and *I. melanopoda* elsewhere in southern Georgia and southeastern Alabama could be important in clarifying the status and nature of *I. junciformis*. Molecular studies of the known and suspected *I. junciformis* populations and of adjacent southern Georgia *Isoetes* populations will likely be necessary to provide a clearer insight into the origins of this apparently rare coastal plain endemic.

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